PATENT COOPERATION TREATY

Translation

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	See form PCT/ISA/210
Applicant's or agent's file reference CP60889	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FR2004/000127	21-01-2004	21-01-2003

International Patent Classification (IPC) or both national classification and IPC
C07K 14/435, C12N 15/11

Applicant
THERAPTOSIS

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/EP	Date of completion of this opinion	Authorized officer
Facsimile No.		Telephone No.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/FR2004/000127

Box No. I **Basis of the report**

1. With regard to the language, this opinion has been established on the basis of:

   ☐ the international application in the language in which it was filed

   ☐ the translation of the international application into _____ , which is the language of a translation furnished for the purposes of international search (Rule 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☐ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE
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International application No.
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Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

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International application No.
PCT/FR2004/000127

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-9	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-9	NO
Industrial applicability (IA)	Claims	1-9	YES
	Claims		NO

2. Citations and explanations:

I. Inventive step (EPC Article 56)

The present application discloses a method for inhibiting the expression of genes coding for ANT isoforms using siRNA specific to said isoforms (example 6 in the present application).

The following documents cited in the international search report are mentioned in the present report; the numbering given below will be used throughout the rest of the procedure:

D1: HALESTRAP ANDREW P ET AL: "The permeability transition pore complex: Another view." BIOCHIMIE (PARIS), vol. 84, no. 2-3, pages 153-166, XP002284573 ISSN: 0300-9084;

D2: VIEIRA H L A ET AL: "Permeabilization of the mitochondrial inner membrane during apoptosis: Impact of the adenine nucleotide translocator" CELL DEATH AND DIFFERENTIATION, vol. 7, no. 12, December 2000 (2000-12), pages 1146-1154, XP009032249 ISSN: 1350-9047;

in the mechanism of apoptosis related to mitochondrial membrane permeabilisation. What is more, D2 mentions that overexpression of the Ant1 isoform induces apoptosis in mammalian cells (page 1150, right-hand column, lines 8 and 9). In addition, D1 specifies that the use of various ANT-regulating drugs will, where necessary, make it possible to protect tissues from cell death caused, in particular, by ischemias or reperfusions or, on the other hand, induce mitochondrial membrane pore opening and thereby cause cell death, for example, as part of chemotherapies.

The gene expression inhibition technique that consists in using siRNA is very frequently used for similar purposes with respect to a plurality of genes (see, for example, documents D1-D4). This technique was used, in particular, by Futami et al. (document D6) to inhibit expression of the *Bcl-2* gene and thereby induce apoptosis (Bcl-2 is an ANT inhibitor).

It follows that the use of such a technique to limit, or inhibit entirely, the expression of ANT isoforms does not appear to involve an inventive step.

Claims 1-9 do not fulfil the requirements of PCT Article 33(3).

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

II. Additional observations

- It should be noted that expressions such as "more particularly", "in particular" and "such as" (claims 1, 4 and 5) do not have any limiting effect on the scope of a claim. As a result, any feature following an expression of this kind is considered to be entirely optional.

- Nucleotide sequences are either homologous or non-homologous. They cannot have a specific degree of *homology* (i.e. "highly homologous"). Two sequences can, on the other hand, have a certain degree of *similarity* or *identity*.

- Contrary to the wording of claim 9, the use of RNAi does not, under any circumstances, induce mitochondrial membrane permeabilisation and cell death because such induction can only be the result of ANT overexpression.

  The only potential effects arising from the use of RNAi are those of limiting the degree of ANT isoform expression and, consequently, limiting mitochondrial membrane permeabilisation and cell death.